Myeloid-Derived Suppressor Cells Express Diverse Biomarkers Across Tumor Type and are Potential Targets for Cancer-Specific and Personalized Immunotherapies

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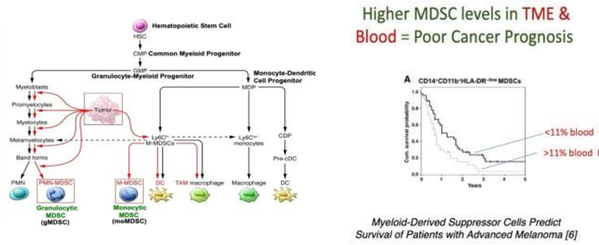
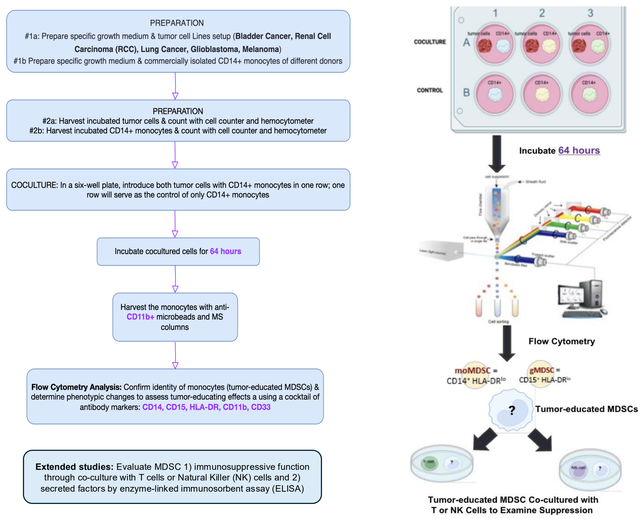
BACKGROUND

Myeloid derived suppressor cells (MDSC) accumulate in the blood and **tumor microenvironments (TME)** of most cancers and correlate with poor cancer prognosis and decreased patient survival [2,6]. In their normal physiological role**, MDSC potently suppress other immune cell such as T and natural killer (NK) cells** in the placenta and wounds [1]. Tumors have 'hijacked' this pathway and produce factors such as prostaglandin E2 [2] that induce myeloid cells to differentiate into MDSC, thereby evading anti-tumor immune responses. Two major types of MDSC have been described: monocytic (**M-MDSC**), expressing higher levels of the CD14 molecule, and granulocytic (**G-MDSC**) [4]. Different cancer types show a *different polarization* to a specific MDSC subset. For example, melanoma is associated with an expansion of M-MDSC, while renal cell carcinoma (RCC) is associated with G-MDSC. Efficacy of T cell cancer immunotherapy as well as chemotherapies are limited because MDSC suppress T cells as well as other immune cells [2,5]. We and our collaborators developed a 'tumor education' model [3] to recapitulate in vivo MDSC generation by co-culturing immune cells – CD14+ monocytes – with tumors to examine MDSC hallmark phenotypic changes such as downregulation of HLA-DR, an antigen presenting molecule, M-MDSC CD14 upregulation as well as soluble suppressive factors and functional suppression of T and NK cells.



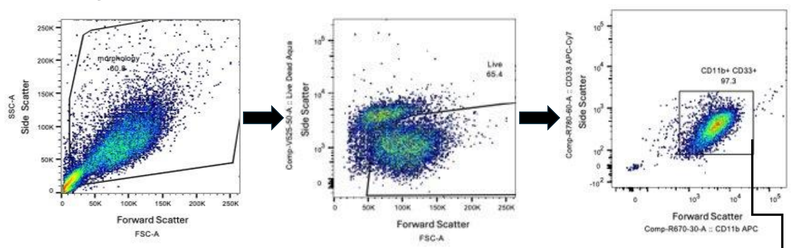
METHODS



RESULTS

Figure 1.

Myeloid Cells Were Recovered from Tumor-Immune Cell Co-cultures

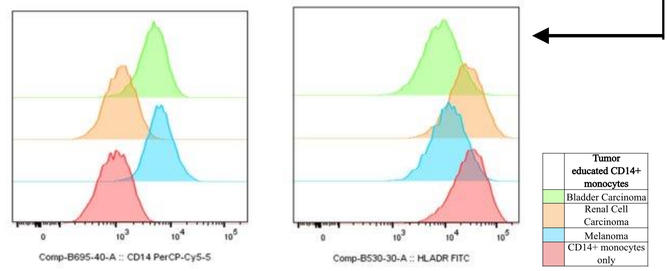


Flow Cytometry Gating Strategy for myeloid cells after tumor 'education.' Forward and side scatter (FSC-A vs. SSC-A) for cell morphology; FSC-A vs. aqua for live cells; and CD33+/CD11b+ to identify myeloid cells.

Figure 2.

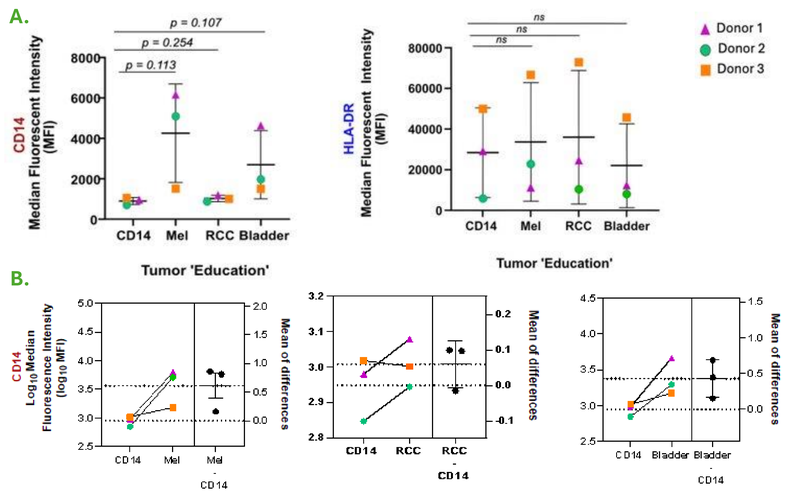
Tumors Except for Renal Cell Carcinoma Induced M-MDSC-Like Cells Decreased HLA-DR and Increased CD14 Expression

Myeloid cells were analyzed for cell surface molecules including CD14 and HLA-DR



CD14 or HLA-DR expression levels of tumor 'educated' myeloid cells for one donor across all tumor types. X-axis, fluorescence intensity; y-axis, % cells. Donor 1 data are shown. Data were analyzed with Flow Jo v.10.5.

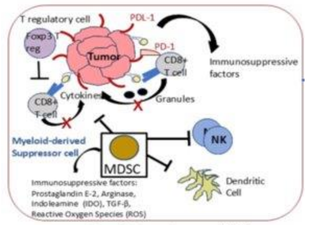
Figure 3. Myeloid Cell CD14 Expression Levels Differ by Tumor Type With Donor-specific Differences in HLA-DR Expression



Changes in myeloid cell CD14 and HLA-DR expression after tumor 'education' for all donors. (A) Each symbol indicates an individual donor's myeloid cells cultured alone (**CD14**) or with: SK-MEL-5 melanoma (**Mel**), 786-O renal cell carcinoma (**RCC**), or T24 adenocarcinoma cells isolated from the bladder (**Bladder**). Statistical analysis: ratio paired t-tests for CD14 monocytes cultured alone and with each tumor type for all donors. (B) The log transformed data, means of MFI differences and standard deviations. ns=not significant; MFI, median fluorescent intensity.

CONCLUSIONS

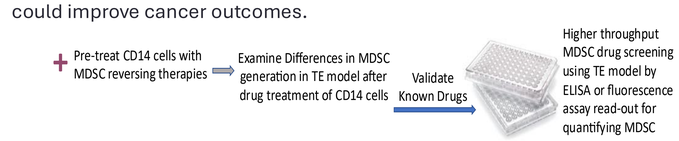
We used the 'tumor education' model to mirror MDSC generation during cancer progression and found that the model recapitulated some aspects of the distinct MDSC populations preferentially expanded in different cancer types. We demonstrate that melanoma induces more M-MDSC-like cells, whereas RCC induces fewer M-MSDC-like cells, consistent with the MDSC expanded in these cancers in patients. Further studies with a larger sample size and assay validation to improve myeloid cell recovery are needed to determine whether



1) these trends continue, and 2) those MDSC-like cells prominently observed after Donor 1 CD14+ monocyte tumor co-cultures, are observed across other donors. We expanded the tumor 'education' model using different tumors in addition to melanoma, including RCC and bladder carcinoma, and to different donors, and observed differences across tumor types and donor, supporting clinical translation of the assay.

FUTURE DIRECTIONS

A hallmark of most cancers is the induction of MDSC, a major immunosuppressive cell type that has normal roles in host immunity but is correlated with poor clinical outcome in most cancers. Due to the importance of MDSC in cancer progression and cancer therapy efficacy there is a critical need for more platforms that evaluate MDSC and MDSC modulating agents. The tumor education model can be used as a prototype to include MDSC in a drug screening assay across tumor types and as a high throughput form for clinical practice. The model proposed here can be adapted to evaluate MDSC responses to anti-cancer drugs and quantify patient MDSC. Developing this approach for screening anti-MDSC therapies and prognostic MDSC patient screening for a range of cancers could improve cancer outcomes.



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